Quant Array (Petroleum) Data Review and Subsequent Actions

Dr. Eleanor M. Jennings
Parsons Corporation

Purpose

- Discuss available ST012 microbial data
- Present subsequent actions
- Goal: Initiate enhanced bioremediation (EBR) pilot study.

Organization of This Talk:

- Review how available microbial data was obtained
- Initial microbial assessment, results, conclusions
- Second microbial assessment (Quant-Array [Petroleum]), results, conclusions
- Subsequent actions

Review of How the Current Microbial Data Set was Obtained

1. In-situ microbial samplers (Bio-Traps®) were used

- Bio-Trap® sampling method: indigenous microbes enter the samplers and get captured for subsequent laboratory analysis

- One sampler installed into each of six MWs
  - Two in the CZ
  - Two in the UWBZ
  - Two in the LSZ

Review of How the Current Microbial Data Set was Obtained (con't)

2. Bio-Trap® samplers retrieved, shipped to laboratory for analysis

3. Analysis performed: quantitative polymerase chain reaction (qPCR)

- Molecular assessment where a known, identified target gene is quantified
- Basic process:
  - Full community DNA is then exposed to a molecular probe designed to target a specific gene (DNA segment)
  - Target gene can be:
    - Functional (example: "anaerobic benzene biodegrader")
    - Identity-based (example: "*Geobacter*")

Review of How the Current Microbial Data Set was Obtained (con't)

- For initial round of qPCR data obtained, two genes targeted for quantification:
  - EBAC: Total eubacterial population
  - APS: Total sulfate-reducing bacteria population

4. Results then obtained

5. The full-community DNA was then frozen by the laboratory. This frozen sample was then used for a second round of qPCR assessments (Quant-Array (Petroleum) analysis)

Initial qPCR Assessment: Results

- Results:

Gene			ST012-CZ20	ST012-LSZ42	ST012-UWBZ31	ST012-LSZ10	ST012-UWBZ24	ST012-CZ02
EBAC	Bacterial population size		2.21 e7	1.6 e5	1.64 e3	5.10 e6	1.33 e6	3.82 e2
APS	SRB bacterial population size		2.41 e6	6.09 e4	ND	ND	2.20 e5	ND

Numbers in YELLOW: Cell or gene counts above the 2.5e2 detection threshold
ND: Not detected above the analysis threshold of 2.5 e2

- Typically, gene counts above the range of 10e5 to 10e6 are considered a substantial population
- EBAC (total population size) gene data interpretation:
  - Measurable population detected in all 6 samples, substantial population size found in 4 samples
- APS (total sulfate-reducing bacteria population) data interpretation:
  - Measurable population found in 3 samples (one from each hydrologic zone), substantial population in 2 samples

Second Microbial Assessment: Quant-Array (Petroleum) Analysis

- Commercially-available test
- 22 genes tested simultaneously are a pre-determined selection of genes commonly found at petroleum-impacted sites, including:
    - Total bacterial population
    - Sulfate-reducing bacteria population
    - Aerobic aromatic hydrocarbon biodegradation (not specific to benzene)
    - Anaerobic aromatic hydrocarbon biodegradation (not specific to benzene)
    - Anaerobic, benzene-specific biodegradation
        - One gene tested, for one pathway that occurs under nitrate-reducing or possibly iron-reducing conditions
- Not all genes involved in benzene-specific biodegradation are tested for in this analysis, thus this analysis does not answer all questions about who, or what capabilities, are present in a sample.

Quant Array (Petroleum) Results: Benzene-Degradation-Related Genes

Gene	Name	Description		ST012-CZ20	ST012-LSZ42	ST012-UWBZ31	ST012-LSZ10	ST012-UWBZ24	ST012-CZ02
TOD	toluene/benzene dioxygenase	Aerobic; Opens aromatic ring for toluene, benzene, and xylenes		4.9 e2	ND	ND	ND	ND	ND
PHE	phenol hydroxylase	Aerobic; Opens aromatic ring for toluene, benzene, and xylenes		2.79 e3	ND	ND	7.99 e2	ND	ND
RDEG	toluene 2 monooxygenase / phenol hydroxylase	Aerobic; Adds oxygen group to the ring of benzene, toluene, xylenes		1.38 e3	ND	ND	3.31 e3	ND	ND
RMO	toluene ring hydroxylating monooxygenase	Aerobic; Adds oxygen group to the ring of benzene, toluene, xylenes		ND	ND	ND	ND	ND	ND
BCR	Benzoyl Coenzyme A Reductase	Anaerobic; Involved in a key step of aromatic compound biodegradation		4.37 e3	ND	ND	ND	ND	ND
BSS	Benzylsuccinate Synthase	Anaerobic, aromatic compound biodegradation		ND	ND	ND	ND	ND	ND
ABC	Benzene Carboxylase	Anaerobic; Initiates anaerobic (nitrate-reducing, maybe iron-reducing) benzene biodegradaton pathway		ND	ND	ND	ND	ND	ND
EBAC	Total Eubacteria	Total microbial population size		2.21 e7	1.6 e5	1.64 e3	5.10 e6	1.33 e6	3.82 e2
APS	Sulfate Reducing Bacteria	Total population size of sulfate-reducing bacteria		2.41 e6	6.09 e4	ND	ND	2.20 e5	ND

Numbers in YELLOW: Cell or gene counts above the 2.5e2 laboratory detection threshold
Numbers in GREEN: Cell or gene counts possibly related to benzene biodegradation, which are also above the 2.5 e2 detection threshold
ND: Not detected above the analysis detection threshold of 2.5 e2

- Typically, gene counts above the range of 10e5 to 10e6 are considered a substantial population

Quant-Array (Petroleum) Analysis: Results and Conclusions

- Genes involved in aerobic aromatic hydrocarbon biodegradation detected in 2 samples
- 1 gene (RDEG) involved in aerobic aromatic hydrocarbon biodegradation was of a substantial population size in 1 sample
- 1 gene (BCR) involved in anaerobic aromatic hydrocarbon biodegradation detected in 1 sample, not of a substantial population size
- Single tested gene (ABC) for benzene-specific biodegradation was not detected
- Not all anaerobic benzene-degrading processes are tested for in this analysis

Subsequent actions

- Initiate enhanced bioremediation (EBR) pilot study.
  - Begin sulfate injections
  - Multiple monitoring rounds to assess microbe growth
  - Assess contaminants of concern concentrations
  - Assess pumping and circulation patterns
  - Optimize pump and circulation patterns
- Evaluate groundwater extraction and treatment contingency to enhance mass removal
- Evaluate bioaugmentation contingency